Gozani Lab
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Histone extraction protocol

1. Harvest cells and wash twice with ice-cold PBS. PBS can be supplemented with 5mM Sodium Butyrate to retain levels of histone acetylation.

2. Resuspend cells in Triton Extraction Buffer (TEB: PBS containing 0.5% Triton X 100 (v/v), 2mM phenylmethylsulfonyl fluoride (PMSF), 0.02% (v/v) NaN3) at a cell density of 107 cells ml-1.

3. Lyse cells on ice for 10 minutes with gentle stirring.

4. Centrifuge at 2000rpm for 10 minutes at 4oC. Remove and discard the supernatant.

5. Wash the cells in half the volume of TEB and centrifuge at before.

6. Resuspend the pellet in 0.2N HCl at a cell density of 4×10^7cells ml-1.

7. Acid extract the histones over night at 4oC.

8. Centrifuge samples at 2000rpm for 10 minutes at 4oC.

9. Removed the supernatant and determine protein content using the Bradford assay.

10. Store aliquots at -20oC.

H-Lysis solution:
0.25M sucrose
3 mM CaCl2.
1 mM Tris pH8.0
0.5% NP-40
Filter sterilize, store at 4oC.

H-Wash solution:
300 mM NaCl.
5 mM MgCl2.
5 mM DTT
0.5% NP-40

H-Extraction solution:
0.5 M HCl
10% glycerol
0.1 M 2-mercaptoethylamine-HCl.

Histone Extraction

(Scully lab method 8/2/01)

1. Wash cells (10^6-10^7) in ice-cold PBS.
2. Add H-Lysis solution.
3. Scrape cell into Eppendorf tube.
4. (Optional: Dounce 20-25 strokes to remove cytoplasmic debris)
5. Spin nuclei to bottom of Eppendorf tube by brief pulse (need to reach top speed. +/- check under microscope). Or spin at 3900 rpm for 5 min.
6. Remove S/N, leaving pellet of nuclei 5-50 µl.
7. Wash pellet with H-Wash solution.
8. Spin again ~3s pulse (Or spin at 3900 rpm for 5 min) as for step 5.
9. Re-suspend nuclear pellet in 3 vol. (15-150 µl) of H-Extraction solution.
10. Leave on ice <=30 min.
11. Spin 13,000 rpm for 5 min at 4°C.
12. Take S/N to new tube.
13. Add 10 Vol. of Acetone.
14. Leave at -20°C for O/N.
15. Spin out precipitate, resuspend in 200 µl sample buffer. Ready for protein gel (5-10 µl for mini gel).